Random/Biased
Region

Primer 1 {

Bgl II

5' LTR

Psi+

Bam HI

SV40 puromycin

3' LTR

pBabe-puro

Not I

amp

ori

Primer 2

- PCR pBabe-puro with
  Primers 1 and 2.
- Cut PCR with Bgl II and Not I.
- Isolate PCR fragment(s) containing
  randomized region from primer 1.

- Cut pBabe-puro with
  Bam HI and Not I.
- Isolate Bam/Not fragment
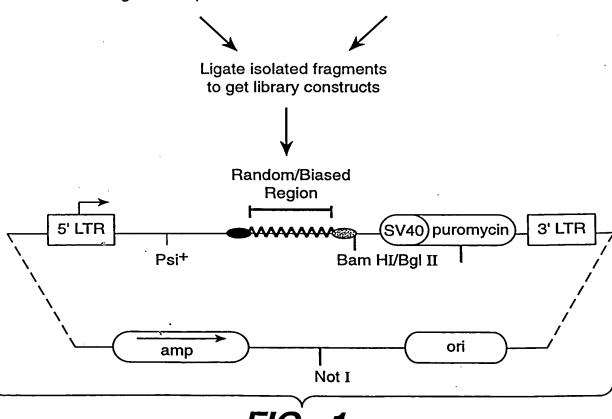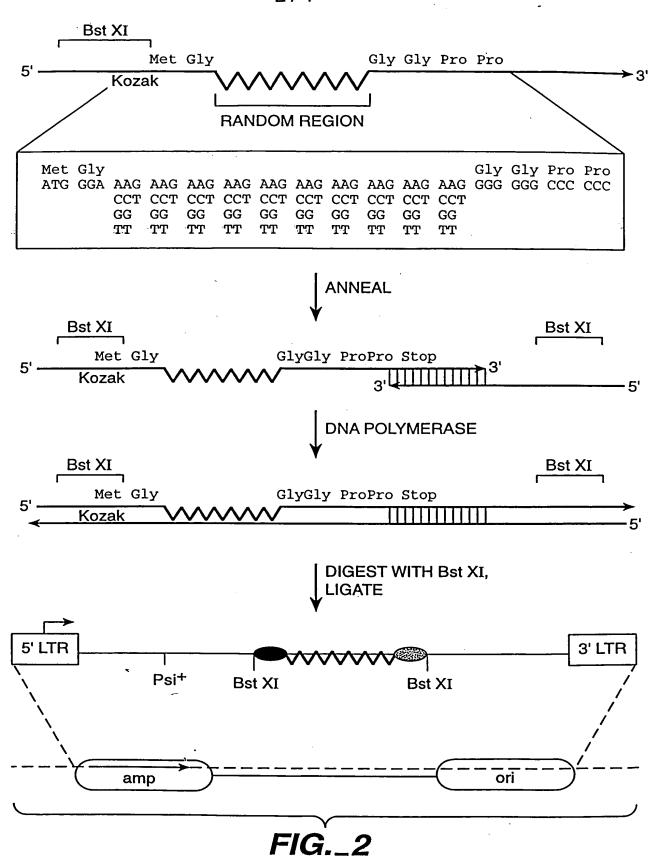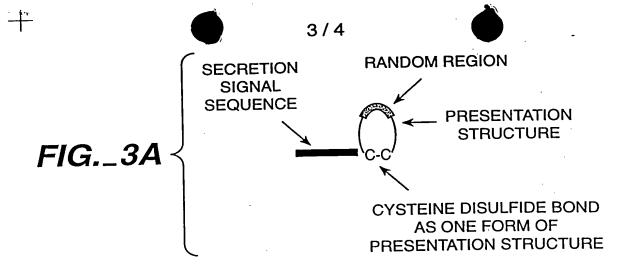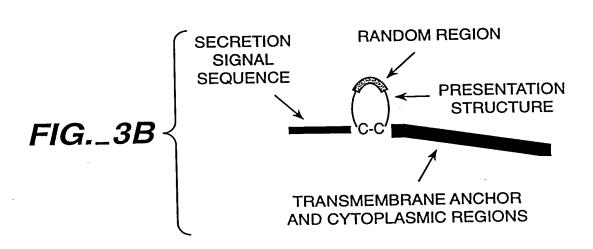 with SV40-puro, 3'LTR, and
  bacterial ori.

Ligate isolated fragments
to get library constructs

Random/Biased
Region

5' LTR

Psi+

Bam HI/Bgl II

SV40 puromycin

3' LTR

amp

ori

Not I

FIG._1

Bst XI

Met Gly   Gly Gly Pro Pro

5'   Kozak   RANDOM REGION   3'

Met Gly											Gly Gly Pro Pro
ATG GGA	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	GGG GGG CCC CCC
	CCT	CCT	CCT	CCT	CCT	CCT	CCT	CCT	CCT	CCT	
	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	
	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	

ANNEAL

Bst XI   Bst XI

Met Gly   GlyGly ProPro Stop

5'   Kozak   3'

3'   5'

DNA POLYMERASE

Bst XI   Bst XI

Met Gly   GlyGly ProPro Stop

5'   Kozak

5'

DIGEST WITH Bst XI,
LIGATE

5' LTR   3' LTR

Psi+   Bst XI   Bst XI

amp   ori

FIG._2

FIG._3A

SECRETION
SIGNAL
SEQUENCE

RANDOM REGION

PRESENTATION
STRUCTURE

C-C

CYSTEINE DISULFIDE BOND
AS ONE FORM OF
PRESENTATION STRUCTURE

FIG._3B

SECRETION
SIGNAL
SEQUENCE

RANDOM REGION

PRESENTATION
STRUCTURE

C-C

TRANSMEMBRANE ANCHOR
AND CYTOPLASMIC REGIONS

FIG._3C

SECRETION
SIGNAL
SEQUENCE

RANDOM REGION

PRESENTATION
STRUCTURE

C C

TRANSMEMBRANE ANCHOR
AND CYTOPLASMIC REGIONS

NheI (29)

LTR (5')

TATA (342)

KpnI (403)

SD (576)

SpeI (562)

Ψ
PACKAGING
SIGNAL

ATG* (984)

BglII (1411)

EcoRI (1423)

BstXI (1428)

NcoI (1434)

NruI (1442)

XhoI (1448)

BstXI (1456)

BamHI (1477)

ClaI (1510)

NheI (1585)

pA site (1898)

KpnI (1959)

XmnI (2197)

PEPTIDES ARE
INSERTED HERE

LTR (3')

pMSCV-RETROVIRUS
PEPTIDE PRESENTATION
CONSTRUCT

BspHI (4997)

BspHI (4389)

BspHI (4284)

XmnI (4044)

ScaI (3927)

BspHI (3276)

FIG._4